Cardioprotection Afforded by SUR2A: An Update

Sofija Jovanović, Khaja Shameem Mohammed Abdul and Aleksandar Jovanović*

Division of Cardiovascular and Diabetic Medicine, Medical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK

Address for Correspondence
Aleksandar Jovanović, Medical Research Institute, Ninewells Hospital & Medical School, University of Dundee, Dundee, DD1 9SY, Scotland, UK, Tel: +44 (0) 1382 496 269; Fax: +44 (0) 1382 632 597; E-mail: a.jovanovic@dundee.ac.uk

Keywords: SUR2A; ATP-sensitive K+ channels; Cardioprotection

Abstract

SUR2A is an atypical ABC protein serving as a regulatory subunit of ATP-sensitive K+ (KATP) channels. In experimental animals, it has been found that an increase in myocardial level of this protein protects the heart against different types of metabolic stresses, including ischaemia. Increase in SUR2A leads to increase in number of fully-assembled KATP channels, which is associated with earlier channel activation during ischaemia as well as increased production of ATP by enzymes that are physically associated with the channel subunits. Activation of KATP channels shorten action membrane potential to prevent Ca2+ influx while ATP production increases subsarcolemmal ATP levels providing energy for vital energy-dependent processes at this localisation. The most recent findings are that SUR2A might also regulate expression of genes that are important in cardioprotection. How to increase SUR2A expression in an efficient and safe manner has been considered. Two possible approaches have been found to be promising. One is gene therapy approach with adenovirus containing SUR2A which was successful at the cellular level and the other was oral nicotinamide, a form of vitamin B3, which was efficient under ex vivo conditions. Based on all these findings, we believe that SUR2A-based strategies against heart ischaemia deserve to be further elucidated and tested. A therapy of ischaemic heart disease exploiting endogenous cardioprotective factors, including SUR2A, would be an excellent adjunct to current therapeutic strategies of ischaemic heart disease and other cardiovascular diseases where increase in cardiac resistance to stress would be beneficial.

Introduction

In 1986 Murry and colleagues discovered the phenomenon that is now called ischaemic preconditioning in which a brief period of blood vessel occlusion enhances the survival of cardiac cells exposed to sustained ischaemia by activating signalling pathway that increases cellular resistance to metabolic stress [1]. It is recognised that therapy of heart ischaemia based on endogenous mechanisms of cardioprotection is warranted and that it would be an important addition to current therapeutic strategies [2,3]. In the last decade, we have focused our research on cardioprotective signalling aiming to identify possible factors of cardioprotection that could be exploited clinically. Initial findings [4-6], encouraged us to do more detailed research of ATP-sensitive K+ (K_{ATP}) channels. These channels have been originally uncovered in cardiomyocytes in 1983 [7]. Their defining attributes are that they are selectively permeable for K+ and are regulated by intracellular ATP. As being regulated by ATP, these channels are generally viewed as a link between intracellular metabolism and membrane excitability [8]. These channels are found in many tissues where they play vital physiological roles such as regulation of insulin secretion (in pancreatic β-cells), regulation of vascular tone and many others [8]. Structurally, K_{ATP} channels are composed of pore-forming inward rectifiers, Kir6.1 or Kir6.2, and regulatory, ATP-binding subunits, SUR1, SUR2A or SUR2B. It is generally accepted that four Kir6.x and four SURx are physically associated with each other to form a functional K_{ATP} channel. The properties of these channels are different in various tissues due to the combinations of the subunits forming the channel. As an example, SUR1 and Kir6.2 form the pancreatic type of K_{ATP} channels, SUR2A and Kir6.2 form the cardiac type of K_{ATP} channels, and SUR2B and Kir6.1 form the vascular smooth muscle type of K_{ATP} channels. There are studies suggesting that further diversity may be generated by a combination of more than one type of Kir6.x and SURx subunits within an individual channel [8]. It should be also pointed out that there are K_{ATP} channels localized on subcellular locations, most notable mitochondrias. The full structure and function (even existence) of these channels is still under vigorous debate [9].

In the heart, cardiac sarcolemmal K_{ATP} channels are composed of pore-forming, Kir6.2, and regulatory, SUR2A, subunits. More recent studies have shown that the sarcolemmal K_{ATP} channel protein complex could be composed of more proteins than just Kir6.2 and SUR2A, including Kir6.1, SUR1, and enzymes regulating intracellular ATP levels and glycolysis. Under physiological conditions K_{ATP} channels are normally closed and are activated in ischaemia [10]. In early days of cardiac sarcolemmal K_{ATP} channels it has been suggested that the activation of sarcolemmal K_{ATP} channels mediate preconditioning [11], but that was later contested as it has been suggested that mitochondrial, but not sarcolemmal, K_{ATP} channels mediated ischemic preconditioning [12,13]. However, later research on transgenic mice lacking K_{ATP} channels demonstrated that they are indeed involved in preconditioning [14]. More recent research demonstrated that sarcolemmal K_{ATP} channels are likely end-effectors of preconditioning [15,16]. Thus, there are little doubts nowadays that the activation of these channels is cardioprotective [17].

Their opening is associated with cardioprotection, but with appearance of arrhythmias as well [18]. In the nineties, K_{ATP} channel opening drugs were considered to be promising therapeutics against heart ischaemia. Unfortunately, their potential use was impeded by extra-cardiac adverse effects (such as hypotension induced by activation of these channels in vascular smooth muscle) and danger of arrhythmias [19]. However, our research in the last decade suggested that there is a possibility to exploit therapeutic properties of sarcolemmal K_{ATP} channels in a much safer way. We have found that levels of SUR2A is a rate limiting factor in forming K_{ATP} channels [6,20] and that increase in expression of SUR2A increases the number of fully assembled K_{ATP} channels [21,22] and confers cardioprotection.

Significantly, an increase of SUR2A was not associated with any cardiac and extracardiac adverse effects and heart electrophysiology was not affected despite increased number of K_{ATP} channels [20,23]. These findings prompted us to study further SUR2A-mediated cardioprotection. In the last few years, we have been particularly focused on 1) mechanism of cardioprotection afforded by SUR2A, and 2) finding ways of up-regulating SUR2A in the heart that could be clinically applicable.

The Mechanism of SUR2A-Mediated Cardioprotection

First original studies on transgenic mice suggested that increased expression of SUR2A results in increased numbers of fully assembled K_{ATP} channels [20] resulting in earlier activation of sarcolemmal K_{ATP} channels when cardiomyocytes were exposed to metabolic stress. How and why and increase in SUR2A alone results in increased levels of K_{ATP} channels is still intriguing. One possibility is that SUR2A is the least expressed K_{ATP} channel subunit in the heart and this has been documented at the mRNA level [6,20]. Being the least expressed channel-forming protein the level of this subunit is possibly a rate limiting factor for making fully-assembled K_{ATP} channels. Indeed, an increase in Kir6.2 levels alone does not have cardioprotective properties as an increase in SUR2A alone does [22]. Timing of activation of K_{ATP} channels in response to ischaemia in cells overexpressing SUR2A resembled closely those seen in preconditioning [15,16,20]. How increased number of K_{ATP} channels transposed into earlier activation of these channels is yet to be fully understood, but the magnitude of K^+ currant flowing through K_{ATP} channels induced by increased probability of channels opening in response to ischaemia would be higher in cells expressing more channels. This means that channel probability would be 0 and >0 in cells with lower and higher channel levels respectively at the beginning of ischaemia, thus transposing to different times of increase in K^+ current. The other possibility is that the higher expression of channels would result in more channels sitting near the site of ATP consumption (membrane ATPases as an example) and therefore would respond earlier to metabolic stress [24]. The activation of K_{ATP} channels shortens action membrane potential duration and prevents influx of Ca^{2+} and Ca^{2+} overload [9]. However, more recent studies suggested that additional mechanisms of SUR2A-mediated cardioprotection independent from the channel activity might be involved. Specifically, sarcolemmal K_{ATP} channels could produce ATP by virtue of catalytic activities of CK and glycolytic enzymes [10]. It is possible that, during metabolic stress, ATP produced by sarcolemmal K_{ATP} channels counteracts stress-induced loss of ATP, which promotes cellular survival. It seems that maintaining subsarcolemmal levels of ATP is particularly valuable effect of K_{ATP} channels in terms of cellular protection as it provides energy at localisation where it is needed most for ion homeostasis [22,25,26]. It has been show that an increase in SUR2A expression in cardiomyocytes increases subsarcolemmal levels of ATP which is cytoprotective independently from the channels activity [22]. Most recently, we have also found one unexpected effect of SUR2A that could have profound consequences on the cellular resistance to stress. Specifically, in experiments performed on embryonic hearts, it was shown that increased expression of SUR2A can shift cardiomycoytes towards less differentiated state by inhibiting extracellular signal-regulated kinases (ERK) signalling pathway and regulating expression of different genes [27]. Whether SUR2A has similar effect on adult cardiomyocytes is yet to be determined. However, the fact that SUR2A inhibits ERK pathway suggests that this is a real possibility. Thus, it

is fair to say that, at the moment, there are 3 possible mechanisms involved in SUR2A-mediated cardioprotection that are not mutually exclusive and are likely complementary to each other:

1) Earlier activation of K_{ATP} channels leading to shortening of action membrane potential and preventing influx of Ca^{2+}.

2) Production of ATP resulting in more energy for energy-dependent processes in subsarcolemmal localisation.

3) Inhibits of ERK signalling pathway with various cardioprotective targets.

It is unlikely that production of ATP is associated with K_{ATP} channels activation as ATP is inhibitor of the channel opening, while channels are more active during ishaemia in SUR2A overexpressing cells that in cells with physiological levels of SUR2A; it seems that the activation of these channels is probably mediated by glycolytic products possessing channel opening properties [25,26,28,29]. Whether ERK signalling pathway is associated in any way with changes in K_{ATP} channels number and activity is yet to be seen.

An Increase of SUR2A in the Heart by Potentially Clinically Applicable Strategies

In early studies, it has been shown that increase in SUR2A can be induced by estrogens or mild hypoxia [30,31]. However, both estrogens and hypoxia are not ideal for clinical use as they may be associated with adverse effects unrelated to SUR2A. In addition, estrogens would likely be efficient only in females. Therefore, we have searched for strategy of increasing SUR2A that could be clinically more applicable. The most obvious approach is gene therapy approach. Gene therapy is based on introduction of a specific gene into somatic cells in order to achieve a therapeutic effect. This concept is now well proven and the main technical obstacles in gene therapy implementation seem to be overcome. Problems with efficacy of gene delivery, safety of vectors and longevity of gene expression seems to be largely resolved and gene therapy approach in therapy of cardiovascular diseases is promising and potentially clinically viable [32]. The most recent types of viral constructs have been developed to secure a long-term expression of a gene in the heart after a single episode of its administration [33]. We have demonstrated that introduction of adenovirus containing SUR2A induced an increase in SUR2A expression, number of functional K_{ATP} channels and resistance to severe metabolic stress in rat heart embryonic H9C2 cell line [22]. Furthermore, we have generated SUR2A in lentiviral vector (LV-SUR2A). Lentivirus is now well established tool to secure long term expression of a gene of interest. Therefore, it seems that LV-SUR2A could be a suitable construct to achieve long-lasting expression of SUR2A in the heart. Another challenge is to establish the most suitable way to introduce LV-SUR2A construct into the heart. There are many methods for gene introduction to the heart and they need to be tested in the context of SUR2A. One promising methods of gene delivery into the heart by using coronary sinus retrograde injection employing non-invasive catheterization has been recently described. This methodology provides a widespread and uniform expression of a gene throughout the ventricular cardiac tissue and seems to be an efficient and safe method for whole heart cardiac gene transfer [34]. Based on all these, it is quite possible that LV-SUR2A introduced into the heart by retrovenous injection in the coronary sinus employing the catheterization technique [34] could generate cardiac phenotype

with permanently increased heart resistance to different types of metabolic stresses.

Another possible clinical strategy could be less invasive and it is based on oral nicotinamide. A decade ago, we have found that a mild chronic hypoxia up-regulates SUR2A due to changes in intracellular NADH/NAD+ ratio [31]. These findings prompted us to consider what kind of treatment might induce a similar effect in the heart and up-regulate SUR2A *in vivo*. It has been reported that a nicotinamide-rich diet could change the NADH/NAD+ ratio, although this has not been demonstrated in the heart [35]. Nicotinamide is the amide form of vitamin B3 (niacin) and is obtained through synthesis in the body or as a dietary source and supplement. Nicotinic acid is the other form of the water soluble vitamin B3. We have specifically choose to test the effect of nicotinamide over more popular nicotinic acid as nicotinamide has no anti-lipid and vascular effects [36]. If a change in intracellular NADH/NAD+ ratio up-regulates SUR2A and K_{ATP} channels in cardiac cells [35], then it was possible that a nicotinamide-rich diet would increase myocardial SUR2A/K_{ATP} channels and, consequently, heart resistance to metabolic stress. Therefore, we have tested this hypothesis by feeding mice for a week with nicotinamide. We have measured expression of SUR2A in the heart, size of myocardial infarction in response to ischaemia reperfusion, physical endurance and survival of isolated cardiomyocytes in severe hypoxia and β-adrenergic stress [37,38]. Details of methods used were described by Sukhodub A et al. [37,38]. We have found that oral nicotinamide significantly decreases infarct size *ex vivo* in response to ischaemia-reperfusion, increases physical endurance and increase cellular survival to severe hypoxia and β-adrenergic stress [37,38]. The maximal effect was observed when daily intake was 2 mg of nicotinamide or below [37,38]. Nicotinamide-induced increase in the expression of SUR2A in the heart and the cardioprotective effect was largely inhibited by HMR1098, an inhibitor of sarcolemmal K_{ATP} channels [37], and in mice unable to form K_{ATP} channels [38]. The maximal effective cardioprotective dose in mice we used is the dose that is equivalent to 500 mg/day of nicotinamide in people or less (the human equivalent dose was calculated using mouse dose and dose translation according to Reagan-Shaw S et al. [39]). Nicotinamide has a great potential as a SUR2A-based therapeutic as it is known to be one of the safest drugs in clinical practice. It should not be confused with nicotinic acid (also known as niacin, vitamin B3 and vitamin PP) that is also a vitamin B3 form but exhibits more pharmacological and adverse effects than nicotinamide [36]. Niacin (nicotinic acid) is known to increase levels of HDL cholesterol, which has been suggested to decrease the risk of cardiovascular events. However, in a recent trial AIM-HIGH, a slow-release form of niacin, used for its effect on lipids, was found to have no effect on cardiovascular event and the trial was halted prematurely on evidence that niacin addition to statins actually increased stroke risk in this group [40]. It is important to point out that in contrast to nicotinic acid nicotinamide has no lipid and vascular effects, that it is not a compound used in AIM-HIGH trial and no similar effect of nicotinamide would be expected [36]. Thus, cardioprotection afforded by nicotinamide has never been tested *in vivo* so far. Current therapeutic use of nicotinamide is confined to treatment of acne vulgaris, diabetes mellitus and head and neck cancer, all of which are in doses above 500 mg/day [36]. It has been suggested that chronic intake of nicotinamide below 3000 mg/day is safe and virtually devoid of adverse effects [41]. Therefore, chronic treatment with 500 mg/day of nicotinamide is likely to be safe. At the same time, it is realistically to expect that nicotinamide taken orally 500 mg/day would significantly increase myocardial resistance to ischaemia. As contraindications and drugs interactions of nicotinamide are rare, almost any patient suffering from IHD on any therapy could use oral nicotinamide for his/her clinical benefit.

Conclusions and Future Perspectives

Increase in expression of SUR2A increases heart resistance towards different types of metabolic stresses, including ishaemia and ischaemia-reperfusion in different animal experimental models. Gene therapy and nicotinamide were identified as two ways of increasing myocardial SUR2A that could be clinically applicable. More animal studies are required to fully establish the mechanism underlying SUR2A-mediated cardioprotection and elucidate *in vivo* efficacy and safety. Such work would provide rationale and justification for clinical trials that would test a clinical value of SUR2A-based therapy.

References

1. Murry, CE, Jennings RB, Reimer KA (1986) Precoditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74: 1124-1136.

2. Edwards RJ, Saurin AT, Rakhit RD, Marber MS (2000) Therapeutic potential of ischaemic preconditioning. Br J Clin Pharmacol 50: 87-97.

3. Hausenloy DJ, Yellon DM (2009) Preconditioning and postconditioning: underlying mechanisms and clinical application. Atherosclerisis 204: 334-341.

4. Jovanović S, Jovanović A (2001) Pinacidil prevents membrane depolarisation and intracellular Ca2+ loading in single cardiomyocytes exposed to severe metabolic stress. Int J Mol Med 7: 639-643.

5. Jovanović S, Jovanović A (2001) Delivery of genes encoding K(ATP) channel subunits in conjuction with pinacidil prevents membrane depolarisation in cells exposed to chemical hypoxia-reoxygenation. Biochem Biophys Res Commun 282: 1098-1102.

6. Ranki HJ, Budas GR, Crawford RM, Jovanović A (2001) Gender-specific difference in cardiac ATP-sensitive K+ channels. J Am Coll Cardiol 38: 906-915.

7. Noma A (1983) ATP-regulated K+ channels in cardiac muscle. Nature 305: 147-148.

8. Nichols CG (2006) KATP channels as molecular sensors of cellular metabolism. Nature 440: 470-476.

9. Garlid KD, Halestrap AP (2012) The mitochondrial K(ATP) channel--fact or fiction? J Mol Cell Cardiol 52: 578-583.

10. Alekseev AE, Hodgson DM, Karger AB, Park S, Zingman LV, et al. (2005) ATP-sensitive K+ channel channel/enzyme multimer: metabolic gating in the heart. J Mol Cell Cardiol 38: 895-905.

11. Gross GJ (1995) ATP-sensitive potassium channels and myocardial preconditioning. Basic Res Cardiol 90: 85-88.

12. Liu Y, Sato T, Seharaseyon J, Szewczyk A, O'Rourke B, et al. (1999) Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. Ann N Y Acad Sci 874: 27-37.

13. Sato T, Marban E (2000) The role of mitochondrial K(ATP) channels in cardioprotection. Basic Res Cardiol 95: 285-289.

14. Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, et al. (2002) Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice. J Clin Invest 109: 509-516.

15. Budas GR, Jovanović S, Crawford RM, Jovanović A (2004) Hypoxia-induced preconditioning in adult stimulated cardiomyocytes is mediated by the opening and trafficking of sarcolemmal KATP channels. FASEB J 18: 1046-1048.

16. Sukhodub A, Jovanović S, Du Q, Budas GR, Clelland A, et al. (2007) AMP-activated protein kinase mediates preconditioning in cardiomyocytes by regulating activity and trafficking of sarcolemmal ATP-sensitive K(+) channels. J Cell Physiol 210: 224-236.

17. Jovanović A, Jovanović S (2009) SUR2A targeting for cardioprotection? Curr Opin Pharmacol 9: 189-193.

18. Quast U (1992) Potassium channel openers: pharmacological and clinical aspects. Fundam Clin Pharmacol 6: 279-293.

19. McPherson GA (1993) Current trends in the study of potassium channel openers. Gen Pharmacol 24: 275-281.

20. Du Q, Jovanović S, Clelland A, Sukhodub A, Budas G, et al. (2006) Overexpression of SUR2A generates a cardiac phenotype resistant to ischemia. FASEB J 20: 1131-1141.

21. Ranki HJ, Crawford RM, Budas GR, Jovanović A (2002) Ageing is associated with decrease in number of sarcolemmal ATP-sensitive K+ channels in a gender-dependent manner. Mech Ageing Dev 123: 695-705.

22. Du Q, Jovanović S, Sukhodub A, Jovanović A (2010) Infection with AV-SUR2A protects H9C2 cells against metabolic stress: a mechanism of SUR2A-mediated cytoprotection independent from the K(ATP) channels activity. Biochim Biophys Acta 1803: 405-415.

23. Sudhir R, Sukhodub A, Du Q, Jovanović S, Jovanović A (2011) Ageing-induced decline in physical endurance in mice is associated with decrease in cardiac SUR2A and increase in cardiac susceptibility to metabolic stress: therapeutic Prospects for up-regulation of SUR2A. Biogerontology 12: 147-155.

24. Zingman LV, Zhu Z, Sierra A, Stepniak E, Burnett CM, et al. (2011) Exercise-induced expression of cardiac ATP-sensitive potassium channels promotes action potential shortening and energy conservation. J Mol Cell Cardiol 51: 72-81.

25. Jovanović S, Du Q, Sukhodub A, Jovanović A (2009) A dual mechanism of cytoprotection afforded by M-LDH in embryonic heart H9C2 cells. Biochem Biophys Acta 1793: 1379-1386.

26. Jovanović S, Du Q, Sukhodub A, Jovanović A (2009) M-LDH physically associated with sarcolemmal K ATP channels mediates cytoprotection in heart embryonic H9C2 cells. Int J Biochem Cell Biol 41: 2295-2301.

27. Land S, Walker D, Du Q, Jovanović A (2013) Cardioprotective SUR2A promotes stem cell properties of cardiomyocytes. Int J Cardiol [Epub ahead of print].

28. Jovanović S, Du Q, Crawford RM, Budas GR, Stagljar I, et al. (2005) Glyceraldehyde 3-phosphate dehydrogenase serves as an accessory protein of the cardiac sarcolemmal K(ATP) channel. EMBO Rep 6: 848-852.

29. Jovanović S, Jovanović A (2005) High glucose regulates the activity of cardiac sarcolemmal ATP -sensitive K+ channels via 1,3-bisphosphoglycerate: a novel link between cardiac membrane excitability and glucose metabolism. Diabetes 54: 383-393.

30. Ranki HJ, Budas GR, Crawford RM, Davies AM, Jovanović A (2002) 17Beta-estradiol regulates expression of K(ATP) channels in heart-derived H9c2 cells. J Am Coll Cardiol 40: 367-374.

31. Crawford RM, Jovanović S, Budas GR, Davies AM, Lad H, et al. (2003) Chronic mild hypoxia protects heart-derived H9c2 cells against acute hypoxia/reoxygenation by regulating expression of the SUR2A subunit of the ATP-sensitive K+ channel. J Biol Chem 278: 31444-31455.

32. Lavu M, Gundewar S, Lefer DJ (2011) Gene therapy for ischemic heart disease. J Mol Cell Cardiol 50: 742-750.

33. Di Pasquale E, Latronico MV, Jotti GS, Condorelli G (2012) Lentiviral vectors and cardiovascular diseases: a genetic tool for manipulating cardiomyocyte differentiation and function. Gene Ther 19: 642-648.

34. Aliño SF, José Herrero M, Bodi V, Noguera I, Mainar L, et al. (2010) Naked DNA delivery to whole pig cardiac tissue by coronary sinus retrograde injection employing non-invasive catheterization. J Gene Med 12: 920-926.

35. Jackson TM, Rawling JM, Roebuck BD, Kirkland JB (1995) Large supplements of nicotinic acid and nicotinamide increase tissue NAD+ and poly(ADP-ribose) levels but do not affect diethylnitrosamine-induced altered hepatic foci in Fischer-344 rats. J Nutr 125:1455-1461.

36. Gille A, Bodor ET, Ahmed K, Offermanns S (2008) Nicotinic acid: Pharmacological effects and mechanism of action. Ann Rev Pharmacol Toxicol 48: 79-106.

37. Sukhodub A, Du Q, Jovanović S, Jovanović A (2010) Nicotinamide-rich diet protects the heart against ischaemia-reperfusion in mice: a crucial role for cardiac SUR2A. Pharmacol Res 61: 564-570.

38. Sukhodub A, Sudhir R, Du Q, Jovanović S, Reyes S, et al. (2011) Nicotinamide-rich diet improves physical endurance by upregulating SUR2A in the heart. J Cell Mol Med 15: 1703-1712.

39. Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22: 659-661.

40. (2011) NIH stops clinical trial on combination cholesterol treatment. In Press releases: The National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health.

41. Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, et al. (2000) Safety of high-dose nicotinamide: a review. Diabetologia 43:1337-1345.